

# Time-resolved two-photon induced anisotropy decay: The rotational diffusion regime

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Two-photon excitation (TPE) of randomly oriented chromophores in solution generates an anisotropic distribution. In a previous paper [Chem. Phys. **179**, 513 (1994)], the polarization dependence of the TPE signal probed by a secondary spectroscopic transition (fluorescence or transient absorption) was determined. In this paper, the time dependence of anisotropic two-photon induced fluorescence or transient absorption signals due to rotational diffusion is treated in spherical tensor formalism. The two-photon signal in general contains isotropic (orientation independent) and anisotropic (orientation dependent) contributions. The latter decay with up to five exponential components. Four time-dependent anisotropy parameters can be defined and measured, allowing additional information, not available in conventional one-photon fluorescence depolarization measurements, to be determined. The special case of one-color TPE is discussed in particular. It is shown that by measurement of the linear and circular anisotropies  $r_1(t)$  and  $r_2(t)$ , more than one rotational correlation time can be determined in many cases, providing information on rotational diffusion parameters not readily determined by analogous one-photon methods and leading in some cases to resolution of rotational motion about the principal diffusion axes of the molecule. © 1994 American Institute of Physics.

## I. INTRODUCTION

Two-photon excitation (TPE) spectroscopy was one of the first new nonlinear spectroscopies to exploit high peak powers available from pulsed lasers<sup>1,2</sup> and has been used to determine the symmetries of excited states and to locate one-photon forbidden states.<sup>3,4</sup> Until recently, however, the dimension of time between the TPE process and a subsequent fluorescence (or transient-absorption) event had not been exploited.

Time-resolved anisotropic TPE measurements were reported recently by Lakowicz and coworkers.<sup>5-7</sup> These measurements demonstrated that initial values of the fluorescence anisotropy were in some cases (linear polyenes, in particular) as high as 0.54. A similar value of the anisotropy was recently measured in our laboratory for the protein bacteriorhodopsin.<sup>8</sup> In other cases, such as indole, the anisotropy was much lower.

The framework for understanding two-photon induced anisotropy values has been given in two recent publications. Callis,<sup>9</sup> basing his treatment on the theory of the polarization dependence of three-photon phenomena developed by McClain,<sup>10</sup> showed that a wide range of anisotropies were possible, depending on the form of the two-photon tensor. The two-photon fluorescence anisotropy values of indole and 3-methylindole were compared to semiempirical predictions and could be understood in at least a qualitative manner. However, the time dependence of the anisotropy was not treated.

Recently, we have developed a theoretical formalism to describe the time dependence of fluorescence or transient absorption following TPE.<sup>11</sup> Spherical tensor formalism was employed to distinguish isotropic and anisotropic components of fluorescence or absorption induced by TPE. We have shown that in the most general case, three isotropic and six

anisotropic two-photon parameters at the initial time ( $t=0$ ) can be measured by time-resolved TPE methods. Four two-photon anisotropy parameters were defined. One of these corresponds to the familiar fluorescence anisotropy, while the other three have no one-photon analog. To obtain these parameters in liquid samples, one must use time-resolved detection of TPE with time resolution shorter than the rotational correlation times. The utility of time-resolved anisotropic TPE is found in this additional information on reorientational dynamics.

The signal in TPE of fluorescence or transient absorption is a function of time delay between excitation and detection. The evolution with time of the signal after TPE could be quite complicated because both rotational decay as well as population decay contribute to the time dependence. The diffusive rotational decay triggered by one-photon excitation contains at most five exponentials. The rotational decay triggered by TPE is more complicated. In our previous paper,<sup>11</sup> we discussed only the simple case of exponential decay. In this paper, we lift the assumption of exponential decay and treat the time dependence of two-photon induced anisotropic fluorescence and transient absorption more generally to derive the time correlation function measured in TPE using the small-step rotational diffusion model. The theoretical description is important to understand the time dependence of fluorescence and transient absorption induced by TPE and to take full advantage of TPE techniques to obtain all the information available concerning the two-photon tensor and reorientational motions of molecules in a randomly oriented system.

In the next section, the orientational averages of the initial anisotropic distribution generated by TPE and of the fluorescence or transient-absorption signal induced are obtained in the rotational diffusion regime. The result is an expression for the time dependence of the isotropic and an-

isotropic components of the TPE signal. In Sec. III, the theory is applied to the usual experimental configuration where a single laser beam is used for TPE. In Sec. IV, the two-photon anisotropy parameters and anisotropy decays are described, and the additional information on rotational dynamics contained in their decays is discussed. Section V presents further discussion and a summary of conclusions.

## II. THEORY

In time-resolved anisotropic two-photon spectroscopy, anisotropic orientational distributions of excited states and ground states are created in the sample system by TPE. These distributions depend on the polarization of the excitation laser beam and the two-photon tensor. Their evolution with time due to both population and reorientational relaxation can be detected by time-resolved anisotropic fluorescence or absorption measurements.

The intensity of the time-dependent anisotropic fluorescence or absorption induced by TPE is given by the orientational average<sup>11</sup>

$$\delta(t) = \langle \langle S_{f0}(0) S_{f0}^*(0) \cdot \mu(t) \mu^*(t) \rangle \rangle K(t), \quad (1)$$

where  $S_{f0}(0)$  is the molecular two-photon tensor at time  $t=0$ ,  $\mu(t)$  is the unit vector of the fluorescence or absorption transition dipole (hereafter called the secondary transition dipole) at time  $t$  after the two-photon excitation,  $K(t)$  is the number density of excited states or of the ground-state hole, and  $\langle \langle \dots \rangle \rangle$  represents the orientational average over the orientations of  $S_{f0}(0)$  and  $\mu(t)$ . Denoting the unit vector of the secondary transition dipole in the laboratory coordinate system by  $\mu = (\mu_x, \mu_y, \mu_z)$ , the orientational average of Eq. (1) can be written as<sup>11</sup>

$$\delta(t) = \sum_{i,j,k,l} \alpha_i \beta_j \alpha_k^* \beta_l^* \lambda \lambda^* \langle \langle S_{ij}(0) S_{kl}^*(0) \mu_\lambda(t) \mu_\lambda^*(t) \rangle \rangle K(t), \quad (2)$$

where  $\alpha$  and  $\beta$  are the unit vectors of the excitation laser polarizations and  $\lambda$  is the unit vector of the polarization of the fluorescence or probe laser beam. The polarization parameters  $\alpha_i \beta_j \alpha_k^* \beta_l^*$  and  $\lambda$  are controlled by the experimentalist.

### A. The orientational distribution

The reorientational motion following TPE can be described by the diffusion equation

$$\frac{\partial f(\Omega, t)}{\partial t} = - \sum_i D_i L_i^2 f(\Omega, t), \quad (3)$$

where the orientational distribution  $f(\Omega, t)$  is defined such that the probability of finding a molecule oriented at  $\Omega$  at time  $t$  is equal to  $f(\Omega, t) d\Omega$ ,  $L_i$  is the angular-momentum operator, and  $D_i$  is the rotational diffusion constant in the principal axis system of the diffusion tensor. The solutions to Eq. (3) have been discussed by a number of authors<sup>12-17</sup> and can be written as

$$f(\Omega, t) = \int f(\Omega_0) \sum_n \Psi_n^*(\Omega_0) \Psi_n(\Omega) \exp(-E_n t) d\Omega_0, \quad (4)$$

where  $f(\Omega_0)$  is the orientational distribution at the initial time  $t=0$ , and  $\Psi_n(\Omega)$ 's are the asymmetric rotor eigenfunctions of the diffusion operator  $-\sum_i D_i L_i^2$  with eigenvalues  $E_n$  determined from

$$- \sum_i D_i L_i^2 \Psi_n = E_n \Psi_n. \quad (5)$$

For higher order terms ( $L > 2$ ), the asymmetric rotor eigenfunctions  $\Psi_n(\Omega)$  cannot be expressed in closed form. However, for terms with  $L \leq 2$ , the asymmetric rotor eigenfunctions can be expanded in the Wigner functions  $D_{m,n}^{(k)}$  (Ref. 18)

$$\Psi_{l,m}^{(L)}(\Omega) = \left( \frac{2L+1}{8\pi^2} \right)^{1/2} \sum_k A_{l,k}^{(L)} D_{m,k}^{(L)*}(\Omega), \quad L \leq 2. \quad (6)$$

In the case of TPE, the initial distribution is proportional to the two-photon absorptivity and can be written as

$$f(\Omega_0) = \sum_{i,j,k,l} \alpha_i \beta_j \alpha_k^* \beta_l^* S_{ij}(\Omega_0) S_{kl}^*(\Omega_0), \quad (7)$$

where  $S_{ij}(\Omega_0)$  is the Cartesian component of the two-photon tensor for molecules oriented at  $\Omega_0$ ;  $\alpha$  and  $\beta$  are the unit vectors of the excitation polarizations; and  $i, j, k$ , and  $l$  denote the laboratory coordinates.

Substituting the initial distribution in Eq. (7) into Eq. (4), we have

$$f(\Omega, t) = \sum_n P_n \Psi_n(\Omega) \exp(-E_n t) \quad (8)$$

with

$$P_n = \sum_{i,j,k,l} \alpha_i \beta_j \alpha_k^* \beta_l^* \int S_{ij}(\Omega_0) S_{kl}^*(\Omega_0) \Psi_n^*(\Omega_0) d\Omega_0. \quad (9)$$

The coefficients  $P_n$  are determined by both the two-photon tensor and the polarizations of the excitation laser beams. The orientational average in Eq. (1) can then be written as

$$\begin{aligned} \delta(t) &= \langle \langle S_{f0}(0) S_{f0}^*(0) \cdot \mu(t) \mu^*(t) \rangle \rangle K(t) \\ &= \frac{3}{8\pi^2} K(t) \int f(\Omega, t) \mu(\Omega) \mu^*(\Omega) d\Omega. \end{aligned} \quad (10)$$

To obtain  $f(\Omega, t)$  and  $\delta(t)$ , the integrations in Eqs. (9) and (10) need to be carried out. This is the subject of the next section.

### B. The orientational average

The orientational average for the initial, zero time delay signal were calculated previously by irreducible spherical tensor formalism.<sup>11</sup> These results are used here. The second-rank tensors  $S_{ij}(\Omega_0)$ ,  $S_{kl}^*(\Omega_0)$ , and  $T_{\lambda\lambda}(\Omega) = \mu_\lambda(\Omega) \mu_\lambda^*(\Omega)$  in laboratory Cartesian coordinates can be

transformed to the spherical tensors  $S_M^{(L)}$ ,  $S_{M'}^{(L')}$ , and  $T_{M''}^{(L'')}$  in molecular coordinates by the transformations

$$S_{ij}(\Omega_0) = \sum_{L=0}^2 \sum_{M=-L}^L \sum_{P=-L}^L U(ij, LP) D_{P,M}^{(L)*}(\Omega_0) S_M^{(L)}, \quad (11)$$

$$S_{kl}^*(\Omega_0) = \sum_{L'=0}^2 \sum_{M'=-L'}^{L'} \sum_{P'=-L'}^{L'} U^*(kl, L' P') D_{P',M'}^{(L')}(\Omega_0) \times S_{M'}^{(L')*}, \quad (12)$$

$$T_{\lambda\lambda}(\Omega) = \sum_{L''=0}^2 \sum_{M''=-L''}^{L''} \sum_{P''=L''}^{L''} U(\lambda\lambda, L'' P'') \times D_{P'',M''}^{(L'')*}(\Omega) T_{M''}^{(L'')}, \quad (13)$$

where  $U(ij, LP)$ 's are the elements of the unitary transformation tabulated elsewhere.<sup>19</sup>

The integration over orientations in Eqs. (9) and (10) is now readily performed by using the orthogonality properties of the Wigner functions. Substitution of Eqs. (11) and (12) into Eq. (9) yields the orientational distribution in Eq. (8) in the form

$$f(\Omega, t) = f^{(0)}(\Omega, t) + f^{(2)}(\Omega, t) + f^{(4)}(\Omega, t), \quad (14)$$

where  $f^{(L)}(\Omega, t)$  is the component of the distribution of rank  $L$ . Not surprisingly, the orientation distribution  $f(\Omega, t)$  contains only even terms of the rotational angular-momentum operator. Since the inversion symmetry is preserved after TPE, as in one-photon excitation, odd  $L$  components in the initial distribution function  $f(\Omega_0)$  must be zero.

Evaluation of the orientational average in this manner was described previously.<sup>11</sup> Following the same procedure, one finds the zero-rank distribution

$$f^{(0)}(\Omega, t) = \sum_{L=0}^2 a_L \delta^{(L)}, \quad (15)$$

where

$$a_L = \sum_{i,j,k,l} \alpha_i \beta_j \alpha_k^* \beta_l^* \frac{1}{2L+1} \times \sum_{P=-L}^L U(ij, LP) U^*(kl, LP), \quad (16)$$

$$\delta^{(L)} = \sum_{M=-L}^L |S_M^{(L)}|^2 \quad (17)$$

and the second-rank distribution function

$$f^{(2)}(\Omega, t) = \sum_{\tau=-2}^2 \sum_{L=0}^2 \sum_{L'=0}^2 \sum_{P''=-2}^2 \sum_{T=-2}^2 H_{P''}^{(LL')} \delta_{\tau}^{(LL')} A_{\tau,T}^{(2)} \times D_{P'',T}^{(2)}(\Omega) \exp(-E_{\tau}^{(2)} t), \quad (18)$$

where

TABLE I. The anisotropic two-photon parameters  $\delta_{\tau}^{(LL')}$ .<sup>a</sup>

$\delta_{\tau}^{(02)} = A_{\tau,0}^{(2)} S_0^{(0)} S_0^{(2)*} + A_{\tau,1}^{(2)} S_0^{(0)} S_1^{(2)*} + A_{\tau,-1}^{(2)} S_0^{(0)} S_{-1}^{(2)*} + A_{\tau,2}^{(2)} S_0^{(0)} S_2^{(2)*} + A_{\tau,-2}^{(2)} S_0^{(0)} S_{-2}^{(2)*}$
$\delta_{\tau}^{(20)} = A_{\tau,0}^{(2)} S_0^{(2)} S_0^{(0)*} - A_{\tau,1}^{(2)} S_1^{(2)} S_0^{(0)*} - A_{\tau,-1}^{(2)} S_{-1}^{(2)} S_0^{(0)*} + A_{\tau,2}^{(2)} S_2^{(2)} S_0^{(0)*} + A_{\tau,-2}^{(2)} S_{-2}^{(2)} S_0^{(0)*}$
$\delta_{\tau}^{(11)} = A_{\tau,0}^{(2)} \frac{1}{\sqrt{6}} (-S_{-1}^{(1)} S_{-1}^{(1)*} + 2S_0^{(1)} S_0^{(1)*} - S_1^{(1)} S_1^{(1)*})$ $+ A_{\tau,1}^{(2)} \frac{1}{\sqrt{2}} (S_0^{(1)} S_1^{(1)*} - S_{-1}^{(1)} S_0^{(1)*}) + A_{\tau,-1}^{(2)} \frac{1}{\sqrt{2}} (S_0^{(1)} S_{-1}^{(1)*}$ $- S_{-1}^{(1)} S_0^{(1)*}) - A_{\tau,2}^{(2)} S_{-1}^{(1)} S_1^{(1)*} - A_{\tau,-2}^{(2)} S_1^{(1)} S_{-1}^{(1)*}$
$\delta_{\tau}^{(12)} = A_{\tau,0}^{(2)} \frac{1}{\sqrt{2}} (S_1^{(1)} S_1^{(2)*} - S_{-1}^{(1)} S_{-1}^{(2)*}) + A_{\tau,1}^{(2)} \left( -\frac{1}{\sqrt{6}} S_0^{(1)} S_1^{(2)*} \right.$ $- \frac{1}{\sqrt{2}} S_{-1}^{(1)} S_0^{(2)*} + \frac{1}{\sqrt{3}} S_1^{(1)} S_2^{(2)*} \left. + A_{\tau,-1}^{(2)} \left( \frac{1}{\sqrt{6}} S_0^{(1)} S_{-1}^{(2)*} \right. \right.$ $+ \frac{1}{\sqrt{2}} S_{-1}^{(1)} S_0^{(2)*} - \frac{1}{\sqrt{3}} S_{-1}^{(1)} S_{-2}^{(2)*} \left. - A_{\tau,2}^{(2)} \left( \frac{\sqrt{2}}{\sqrt{3}} S_0^{(1)} S_2^{(2)*} + \frac{1}{\sqrt{3}} S_{-1}^{(1)} S_1^{(2)*} \right) \right.$ $+ A_{\tau,-2}^{(2)} \left( \frac{\sqrt{2}}{\sqrt{3}} S_0^{(1)} S_{-2}^{(2)*} + \frac{1}{\sqrt{3}} S_1^{(1)} S_{-1}^{(2)*} \right)$
$\delta_{\tau}^{(21)} = A_{\tau,0}^{(2)} \frac{1}{\sqrt{2}} (S_1^{(2)} S_1^{(1)*} - S_{-1}^{(2)} S_{-1}^{(1)*}) + A_{\tau,1}^{(2)} \left( -\frac{1}{\sqrt{2}} S_0^{(2)} S_1^{(1)*} \right.$ $- \frac{1}{\sqrt{6}} S_{-1}^{(2)} S_0^{(1)*} + \frac{1}{\sqrt{3}} S_{-2}^{(2)} S_{-1}^{(1)*} \left. + A_{\tau,-1}^{(2)} \left( \frac{1}{\sqrt{2}} S_0^{(2)} S_{-1}^{(1)*} \right. \right.$ $+ \frac{1}{\sqrt{6}} S_{-1}^{(2)} S_0^{(1)*} - \frac{1}{\sqrt{3}} S_2^{(2)} S_1^{(1)*} \left. + A_{\tau,2}^{(2)} \left( \frac{\sqrt{2}}{\sqrt{3}} S_0^{(2)} S_2^{(1)*} \right. \right.$ $+ \frac{1}{\sqrt{3}} S_{-1}^{(2)} S_1^{(1)*} \left. - A_{\tau,-2}^{(2)} \left( \frac{\sqrt{2}}{\sqrt{3}} S_0^{(2)} S_{-2}^{(1)*} + \frac{1}{\sqrt{3}} S_{-1}^{(2)} S_{-1}^{(1)*} \right) \right)$
$\delta_{\tau}^{(22)} = A_{\tau,0}^{(2)} \frac{1}{\sqrt{14}} (2S_2^{(2)} S_2^{(2)*} - S_1^{(2)} S_1^{(2)*} - 2S_0^{(2)} S_0^{(2)*} - S_{-1}^{(2)} S_{-1}^{(2)*})$ $+ 2S_{-2}^{(2)} S_{-2}^{(2)*} + A_{\tau,1}^{(2)} \frac{1}{\sqrt{14}} (-S_0^{(2)} S_1^{(2)*} + S_{-1}^{(2)} S_0^{(2)*} + \sqrt{6} S_{-2}^{(2)} S_{-1}^{(2)*}$ $- \sqrt{6} S_1^{(2)} S_2^{(2)*}) + A_{\tau,-1}^{(2)} \frac{1}{\sqrt{14}} (-S_0^{(2)} S_{-1}^{(2)*} + S_1^{(2)} S_0^{(2)*} + \sqrt{6} S_2^{(2)} S_{-1}^{(2)*}$ $- \sqrt{6} S_{-1}^{(2)} S_{-2}^{(2)*}) + A_{\tau,2}^{(2)} \left( \frac{\sqrt{2}}{\sqrt{7}} S_0^{(2)} S_2^{(2)*} + \frac{\sqrt{2}}{\sqrt{7}} S_{-2}^{(2)} S_0^{(2)*} + \frac{\sqrt{3}}{\sqrt{7}} S_{-1}^{(2)} S_1^{(2)*} \right)$ $+ A_{\tau,-2}^{(2)} \left( \frac{\sqrt{2}}{\sqrt{7}} S_2^{(2)} S_0^{(2)*} + \frac{\sqrt{2}}{\sqrt{7}} S_0^{(2)} S_{-2}^{(2)*} + \frac{\sqrt{3}}{\sqrt{7}} S_1^{(2)} S_{-1}^{(2)*} \right)$

<sup>a</sup>The coefficients  $A_{\tau,T}^{(2)}$  are given in Refs. 12, 13, and 17, and  $S_M^{(L)}$ 's are defined in Eqs. (11) and (12).

$$H_{P''}^{(LL')} = \sum_{i,j,k,l} \alpha_i \beta_j \alpha_k^* \beta_l^* \sum_{P=-L}^L \sum_{P'=-L'}^{L'} (-1)^{-P} C(LL' 2; -PP' P'') U(ij, LP) U^*(kl, L' P') \quad (19)$$

and

$$\delta_{\tau}^{(LL')} = \sum_{M=-L}^L \sum_{M'=-L'}^{L'} \sum_{M''=-2}^2 (-1)^M C(LL' 2j; -MM' M'') S_M^{(L)} S_{M'}^{(L')*} A_{\tau,M''}^{(2)}, \quad (20)$$

where  $C(LL'2; -P\dot{P}'P'')$  and  $C(LL'2; -MM'M'')$  are the Clebsch–Gordan coefficients. The expressions for  $\delta_\tau^{(LL')}$  in Eq. (20), given in Table I, can be compared with the expressions for  $\delta_\tau^{(LL')}$  obtained previously,<sup>11</sup> showing that  $T_{M''}^{(2)}$  is replaced here with the expansion coefficients  $A_{\tau M''}^{(2)}$ . (Expressions for  $\delta_\tau^{(LL')}$  are given in Table III of Ref. 11.)

The fourth-rank distribution function is

$$f^{(4)}(\Omega, t) = \sum_{n, P, P', M, M'} H_{P, P'}^{(4)} S_M^{(2)} S_{M'}^{(2)*} \int d\Omega_0 D_{P, M}^{(2)}(\Omega_0) \times D_{P', M'}^{(2)}(\Omega_0) \phi_n(\Omega_0) \phi_n^*(\Omega) \times \exp(-E_n t), \quad (21)$$

where

$$H_{P, P'}^{(4)} = \sum_{i, j, k, l} \alpha_i \beta_j \alpha_k^* \beta_l^* U(ij, 2P) U^*(kl, 2P'). \quad (22)$$

For higher-rank terms ( $L=4$ ), eigenfunctions can only be obtained for the symmetric-rotor case.<sup>12,14</sup> Fortunately, for TPE-induced fluorescence or absorption, only the zero and second rank distribution functions are required.<sup>11</sup>

The time dependence of fluorescence or absorption induced by TPE can now be obtained by substituting  $f(\Omega, t)$  into Eq. (10), yielding

$$\delta(t) = K(t) \left[ \sum_{L=0}^2 a_L \delta^{(L)} + \sum_{\tau=-2}^2 \sum_{L=0}^2 \sum_{L'=0}^2 b_{LL'} B_\tau \delta_\tau^{(LL')} \right] \times \exp(-E_\tau^{(2)} t), \quad (23)$$

where  $\delta^{(L)}$  are the isotropic two-photon parameters defined in Eq. (17) and  $\delta_\tau^{(LL')}$ 's are the anisotropic two-photon parameters defined in Eq. (20). Expressions for  $\delta_\tau^{(LL')}$  are gathered in Table I. In Eq. (23),  $B_\tau$  is a parameter describing the orientation of the secondary transition dipole and is given by

$$B_\tau = \sum_{m=-2}^2 A_{\tau, m}^{(2)} T_m^{(2)}, \quad (24)$$

where  $T_m^{(2)}$ 's are the spherical components of the transition dipole. The parameters  $a_L$  and  $b_{LL'}$  in Eq. (23) are polarization parameters for the isotropic and anisotropic decays, respectively, and are controlled by experimentalist

$$a_L = \sum_{i, j, k, l} \alpha_i \beta_j \alpha_k^* \beta_l^* B_{ijkl}^{(L)}, \quad (25)$$

$$b_{LL'} = \sum_{i, j, k, l} \alpha_i \beta_j \alpha_k^* \beta_l^* \lambda \lambda^* B_{ijkl, \lambda}^{(LL')}, \quad (26)$$

where the polarization coefficients  $B_{ijkl}^{(L)}$  and  $B_{ijkl, \lambda}^{(LL')}$  were given in Ref. 11 (Table II). (Without loss of generality, we choose  $\mu_\lambda$  to define one laboratory axis;  $\lambda=1$  for that axis and 0 otherwise.)

Equation (23) is the key result of this section and shows that the time evolution of the TPE signal contains two

components—one isotropic and the other anisotropic. The isotropic decay evolves with the population kinetics only and does not depend on rotational motion of the molecules. The anisotropic decay, on the other hand, is rotation dependent and contains as many as five exponentials for an asymmetric rotor. The isotropic decay depends on two parameters—the isotropic two-photon absorptivity  $\delta^{(L)}$  and the isotropic polarization parameter  $a_L$ . There are three isotropic two-photon absorptivity parameters,  $\delta^{(0)}$ ,  $\delta^{(1)}$ , and  $\delta^{(2)}$ , which can be related to McClain's  $\delta$  parameters.<sup>2,11</sup> The anisotropic decay is dependent on three parameters—the anisotropic two-photon absorptivity  $\delta_\tau^{(LL')}$ , the transition dipole orientation parameter  $B_\tau$ , and the anisotropic polarization parameter  $b_{LL'}$ . There are six anisotropic two-photon absorptivity parameters for each exponential decay,  $\delta_\tau^{(02)}$ ,  $\delta_\tau^{(20)}$ ,  $\delta_\tau^{(11)}$ ,  $\delta_\tau^{(12)}$ ,  $\delta_\tau^{(21)}$ , and  $\delta_\tau^{(22)}$ . These anisotropic two-photon parameters have no correspondence to McClain's  $\delta$  parameters. The polarization parameters  $a_L$  and  $b_{LL'}$  are tabulated for several experimental configurations in Ref. 11.

The isotropic and anisotropic two-photon parameters  $\delta^{(L)}$  and  $\delta_\tau^{(LL')}$  and the orientation parameters of the transition dipole  $B_\tau$  are molecular parameters and independent of the experimental polarization;  $\delta^{(L)}$  and  $\delta_\tau^{(LL')}$  are determined by the two-photon tensor; and  $B_\tau$  can be obtained from the orientation of the transition dipole. In contrast, the isotropic and anisotropic polarization parameters  $a_L$  and  $b_{LL'}$  depend on the experimental polarization. It is via this polarization dependence that the abundant information inherent in time-resolved anisotropic two-photon spectroscopy can be extracted. The relative contribution of each exponential component, labeled by  $\tau=-2, -1, 0, 1, 2$ , to the anisotropic part of  $\delta(t)$  depends on the product  $b_{LL'} B_\tau \delta_\tau^{(LL')}$ , which can be viewed as weighting factors determining the relative contribution of each exponential. Since  $b_{LL'}$  depends on the polarization configuration of experiment, the polarization dependence of the anisotropy decay might allow the rotational components to be distinguished by measuring various polarization configurations. A set of independent polarization configurations was discussed in our previous paper.<sup>11</sup>

If the secondary transition dipole lies along one of the molecular axes (say the  $z$  axis), the anisotropic decay in Eq. (23) reduces to two exponentials since  $A_{\tau, 0}^{(2)}$  in Eq. (24) is nonzero only when  $\tau=0$  and 2 for an asymmetric rotor. The rotational decay of  $\delta(t)$  contains only two exponential decays in this case with rotational eigenvalues  $E_0^{(2)}$  and  $E_2^{(2)}$ .

The time evolution of TPE in Eq. (23) is similar to the time dependence in anisotropic fluorescence,<sup>12–16</sup> anisotropic Raman,<sup>20</sup> and anisotropic coherent anti-Stokes Raman Spectroscopy (CARS),<sup>21</sup> where anisotropic decay is triggered by one-photon excitation and similarly contains five exponentials in general. In spite of this similarity, two-photon excitation induces a more complex orientational distribution with higher rank terms ( $L=4$ ), which, while they cannot be detected by a one-photon probing event, might be probed by appropriate methods such as Raman or CARS spectroscopy.

TABLE II. The anisotropic two-photon parameters  $\delta_r^{(LL')}$  for a diagonal two-photon tensor.<sup>a</sup>

$$\begin{aligned}\delta_r^{(02)} &= -\frac{1}{3\sqrt{2}} A_{\tau,0}^{(2)}(S_{xx} + S_{yy} + S_{zz})(2S_{zz} - S_{xx} - S_{yy}) - \frac{1}{2\sqrt{3}} (A_{\tau,2}^{(2)} \\ &\quad + A_{\tau,-2}^{(2)})(S_{xx} + S_{yy} + S_{zz})(S_{xx} - S_{yy}) \\ \delta_r^{(20)} &= \delta_r^{(02)} \\ \delta_r^{(22)} &= \frac{1}{\sqrt{14}} A_{\tau,0}^{(2)} \left[ (S_{xx} - S_{yy})^2 - \frac{1}{3} (2S_{zz} - S_{xx} - S_{yy})^2 \right] \\ &\quad + \frac{1}{\sqrt{21}} (A_{\tau,2}^{(2)} + A_{\tau,-2}^{(2)})(2S_{zz} - S_{xx} - S_{yy})(S_{xx} - S_{yy})\end{aligned}$$

<sup>a</sup>The coefficients  $A_{\tau,T}^{(2)}$  are given in Refs. 12, 13, and 17.

### III. DEGENERATE TWO-PHOTON EXCITATION

TPE can be achieved by two laser pulses with different wavelengths or by a single laser pulse with a single wavelength. In the discussion above, no restrictions were imposed on the two-photon excitation process. Since in practice most TPE experiments use a single laser beam from which two identical photons are absorbed, the case of degenerate TPE is discussed in particular in this section. In this case,  $S_{ij} = S_{ji}$  when  $\nu_\alpha = \nu_\beta$ . The two-photon absorption tensor is symmetric for two photons of equal energy. When the two-photon tensor  $S_{ij}$  is symmetric, the antisymmetric tensor  $S_M^{(1)}$  is zero. Thus, the isotropic parameter  $\delta^{(1)}$  and the anisotropic parameters  $\delta_r^{(11)}$ ,  $\delta_r^{(12)}$ , and  $\delta_r^{(21)}$  vanish. Five nonzero parameters remain for each rotational component  $\tau$ — $\delta_r^{(0)}$ ,  $\delta_r^{(2)}$ ,  $\delta_r^{(02)}$ ,  $\delta_r^{(20)}$ , and  $\delta_r^{(22)}$ . These restrictions simplify the two-photon parameters. In this section, we discuss two specific cases (1) that of a diagonal two-photon tensor and (2) that of a two-dimensional two-photon tensor.

#### A. Diagonal two-photon tensor

If the three principal axes of the two-photon tensor coincide with the principal axes of rotational diffusion, the tensor elements in the molecular axes are reduced to three diagonal tensor elements. If we also assume that the tensor is real (i.e., the intermediate virtual state is nonresonant), then  $\delta_r^{(02)} = \delta_r^{(20)}$ . Thus, the two isotropic two-photon constants  $\delta^{(0)}$  and  $\delta^{(2)}$  and the three anisotropic constants  $\delta_r^{(02)}$ ,  $\delta_r^{(20)}$ , and  $\delta_r^{(22)}$  can be determined from the three tensor elements. The resulting two-photon constants for a diagonal two-photon tensor are shown in Table II.

In the case of a two-photon tensor that is diagonal in the principal axis system of the diffusion tensor, the anisotropic decay contains only two exponentials. This can be seen from Table II, where it is apparent that the anisotropic two-photon parameters  $\delta_r^{(02)}$  and  $\delta_r^{(22)}$  contain only the terms with  $A_{\tau,0}^{(2)}$  and  $A_{\tau,2}^{(2)} + A_{\tau,-2}^{(2)}$ , which are nonzero only when  $\tau=0$  and 2. In this case, the number of exponentials is the same (two) for an asymmetric rotor whether one, two, or three diagonal elements are nonzero. However, the relative amplitude for each component depends on the tensor elements.

TABLE III. The anisotropic two-photon parameters  $\delta_r^{(LL')}$  for a two-dimensional tensor.<sup>a</sup>

$$\begin{aligned}\delta_r^{(02)} &= \frac{1}{3\sqrt{2}} A_{\tau,0}^{(2)}(S_{xx} + S_{yy})^2 - \frac{1}{2\sqrt{3}} (A_{\tau,2}^{(2)} + A_{\tau,-2}^{(2)})(S_{xx}^2 - S_{yy}^2) \\ &\quad + \frac{i}{\sqrt{3}} (A_{\tau,2}^{(2)} - A_{\tau,-2}^{(2)})(S_{xx} + S_{yy})S_{xy} \\ \delta_r^{(20)} &= \delta_r^{(02)} \\ \delta_r^{(22)} &= \frac{1}{\sqrt{14}} A_{\tau,0}^{(2)} \left[ (S_{xx} - S_{yy})^2 + 4S_{xy}^2 - \frac{1}{3} (S_{xx} + S_{yy})^2 \right] \\ &\quad - \frac{1}{\sqrt{21}} (A_{\tau,2}^{(2)} + A_{\tau,-2}^{(2)})(S_{xx}^2 - S_{yy}^2) + i \frac{2}{\sqrt{21}} (A_{\tau,2}^{(2)} \\ &\quad - A_{\tau,-2}^{(2)})(S_{xx} + S_{yy})S_{xy}\end{aligned}$$

<sup>a</sup>The coefficients  $A_{\tau,T}^{(2)}$  are given in Refs. 12, 13, and 17.

#### B. Two-dimensional tensor

Another interesting case is that where the two-photon tensor is taken to be real and to have nonzero elements in only two dimensions. This is a reasonable assumption for most polycyclic aromatic hydrocarbons, which usually have low energy states polarized in the plane of the molecule. If the molecular plane is taken to be in the  $xy$  plane, the nonzero elements of  $S_{ij}$  are  $S_{xx}$ ,  $S_{yy}$ , and  $S_{xy} = S_{yx}$ . The two-photon parameters  $\delta^{(L)}$  and  $\delta_r^{(LL')}$  can be obtained in terms of these tensor elements. In Table III, the parameters  $\delta_r^{(LL')}$  are listed for a two-dimension tensor.

In the case of the two-dimensional tensor, the anisotropic two-photon parameters  $\delta_r^{(02)} = \delta_r^{(20)}$  and  $\delta_r^{(22)}$  contain only the terms with  $\tau=0$  and  $\pm 2$  ( $A_{\tau,0}^{(2)}$  and  $A_{\tau,2}^{(2)} + A_{\tau,-2}^{(2)}$  are nonzero only when  $\tau=0$  and 2, and  $A_{\tau,2}^{(2)} - A_{\tau,-2}^{(2)}$  is nonzero only when  $\tau=-2$ ). Hence the anisotropic decay for this case reduces to at most three exponentials with  $\tau=0$ , 2, and  $-2$ . If the tensor element  $S_{xy}=0$ , the anisotropic two-photon parameters contains only the terms with  $\tau=0$  and 2, and only the two exponentials with  $\tau=0$ , 2 remain, as discussed above for a diagonal two-photon tensor.

### IV. TWO-PHOTON ANISOTROPY DECAY

Perhaps the most significant advantage of time-resolved anisotropic two-photon spectroscopy is that more than one anisotropy parameter can be measured, and this fact may permit multiple decay components to be distinguished in the anisotropy decay. Based on the calculation of the time and polarization dependencies of the two-photon absorptivity above, the two-photon anisotropy decay can now be discussed in general in terms of the following two-photon anisotropies that were described in our previous paper:<sup>11</sup>

$$r_1(t) = \frac{\delta_{1111,1}(t) - \delta_{2222,1}(t)}{\delta_{1111,1}(t) + 2\delta_{2222,1}(t)}, \quad (27)$$

$$r_2(t) = \frac{\delta_{\text{cir},\parallel}(t) - \delta_{\text{cir},\perp}(t)}{\delta_{\text{cir},\perp}(t) + 2\delta_{\text{cir},\parallel}(t)}, \quad (28)$$

$$r_3(t) = \frac{\delta_{1212,1}(t) - \delta_{2121,1}(t)}{\delta_{1212,1}(t) + \delta_{2121,1}(t) + \delta_{2323,1}(t)}, \quad (29)$$

$$r_4(t) = \frac{\delta_{1212,1}(t) - \delta_{2323,1}(t)}{\delta_{1212,1}(t) + \delta_{2121,1}(t) + \delta_{2323,1}(t)}, \quad (30)$$

where

$$\delta_{ijkl,\lambda} = \langle \langle S_{ij}(0) S_{kl}^*(0) \mu_\lambda^*(t) \mu_\lambda(t) \rangle \rangle K(t). \quad (31)$$

In these definitions,  $\delta_{ijkl,\lambda}(t)$  is the signal generated by the two photons with polarizations  $i=k$  and  $j=l$  in laboratory coordinates, and  $\lambda$  is the polarization of the fluorescence or the probe laser beam and is chosen along one of the laboratory coordinate axes. The two-photon absorptivities  $\delta_{\text{cir},\parallel}$  and  $\delta_{\text{cir},\perp}$  are the two-photon absorptivities generated with circularly polarized light and detected with polarization parallel or perpendicular, respectively, to the plane of circular polarization. The parameter  $r_1$  is the linear anisotropy defined analogously to the fluorescence anisotropy, while the circular anisotropy  $r_2$ , as well as the anisotropies  $r_3$  and  $r_4$ , have no corresponding analog in fluorescence and transient absorption induced by one-photon excitation, but rather are similar to anisotropies that can be defined in anisotropic Raman and anisotropic CARS.<sup>20,21</sup>

Expressions for the two-photon absorptivity  $\delta_{ijkl,\lambda}$  and sums and differences yielding, respectively, isotropic and anisotropic components of the two-photon signals have been tabulated (see Tables II and IV of Ref. 11). From these expressions, together with the time dependence of the two-photon absorptivity in Eq. (23), we find

$$r_1(t) = \sum_{\tau=-2}^2 \frac{-\sqrt{3} B_\tau (\delta_\tau^{(02)} + \delta_\tau^{(20)} + (2/\sqrt{7}) \delta_\tau^{(22)})}{5 \delta^{(0)} + 2 \delta^{(2)}} e^{-E_\tau^{(2)} t}, \quad (32)$$

$$r_2(t) = -\frac{\sqrt{3}}{\sqrt{7}} \sum_{\tau=-2}^2 \frac{B_\tau \delta_\tau^{(22)}}{\delta_\tau^{(2)}} e^{-E_\tau^{(2)} t}, \quad (33)$$

$$r_3(t) = \sum_{\tau=-2}^2 \left[ \frac{-2\sqrt{3} B_\tau (\delta_\tau^{(12)} + \delta_\tau^{(21)})}{5 \delta^{(1)} + 3 \delta^{(2)}} \right] e^{-E_\tau^{(2)} t}, \quad (34)$$

$$r_4(t) = \sum_{\tau=-2}^2 \frac{B_\tau \left[ -3 \delta_\tau^{(11)} - \sqrt{3} (\delta_\tau^{(12)} + \delta_\tau^{(21)}) - \frac{3\sqrt{3}}{\sqrt{7}} \delta_\tau^{(22)} \right]}{5 \delta^{(1)} + 3 \delta^{(2)}} \times e^{-E_\tau^{(2)} t}. \quad (35)$$

Although in general these anisotropy decays contain five exponentials, in some cases the number of exponentials is reduced, as discussed in Sec. III. The number of decay components in these anisotropy decays depends on the orientations of the secondary transition dipole and the molecular two-photon tensor, which in turn depend on the molecular structure. The two-photon anisotropies  $r_1$  and  $r_2$  can be detected by single laser excitation, while  $r_3$  and  $r_4$  require two-

color excitation. Since excitation by a single laser in TPE is more common, measurements of  $r_1$  and  $r_2$  anisotropy decays will be discussed here.

As an illustrative example, we consider the anisotropies  $r_1$  and  $r_2$  for a tensor with nonzero elements  $S_{xx}$  and  $S_{yy}$ . All other elements are assumed to vanish and the orientation of the secondary transition dipole is taken to be along the  $x$  axis. In this case,

$$r_1(t) = \frac{(A_{0,0}^{(2)})^2}{7(2+P^2)} \left\{ (1+\sqrt{3}C) \left( 2+P^2 + \frac{9}{\sqrt{3}} PC \right) e^{-E_0^{(2)} t} + (C-\sqrt{3}) \left[ (2+P^2)C - \frac{9}{\sqrt{3}} P \right] e^{-E_2^{(2)} t} \right\} \quad (36)$$

and

$$r_2(t) = \frac{(A_{0,0}^{(2)})^2}{7(1+3P^2)} \left\{ (1+\sqrt{3}C) \left( 3P^2 - 1 + \frac{6}{\sqrt{3}} PC \right) e^{-E_0^{(2)} t} + (C-\sqrt{3}) \left[ (3P^2 - 1)C - \frac{6}{\sqrt{3}} P \right] e^{-E_2^{(2)} t} \right\}, \quad (37)$$

where

$$P = \frac{S_{xx} - S_{yy}}{S_{xx} + S_{yy}}, \quad (38)$$

$$C = \frac{A_{2,0}^{(2)}}{A_{0,0}^{(2)}}. \quad (39)$$

It is apparent that  $r_1$  and  $r_2$  in Eqs. (36) and (37) contain two exponentials and that the relative contributions of each exponential ( $\tau=0,2$ ) in  $r_1$  is different from that in  $r_2$ , depending on the two constants  $P$  and  $C$  defined in Eqs. (38) and (39).  $C$  is a shape factor of molecule;  $C=0$  for a symmetric rotor (with  $D_z > D_x = D_y$ ); for an asymmetric rotor, the values of  $C$  depends on the relative values of  $D_x$ ,  $D_y$ , and  $D_z$ . The constant  $P$  is determined by the relative values of  $S_{xx}$  and  $S_{yy}$ . If  $S_{yy}=0$ , we have  $P=1$ ; if  $S_{yy}=S_{xx}$ ,  $P=0$ ; if  $S_{xx}=0$ ,  $P=-1$ ; and if  $S_{xx}=-S_{yy}$ ,  $P=\infty$ .

Although these considerations show that the  $r_1$  and  $r_2$  anisotropy decays are double exponential, it is likely to be difficult to determine two decay components reliably by fitting the decay of either  $r_1(t)$  or  $r_2(t)$ , just as, in one-photon fluorescence anisotropy decay measurements, only one decay component has been determined for many asymmetric molecules. However, since it is possible to detect two anisotropies  $r_1$  and  $r_2$  in TPE, it should be possible to determine additional rotational decay parameters. Although two anisotropies may not be sufficient to distinguish five exponentials in the most general case, they might suffice to distinguish two exponentials in the case where the secondary dipole is along one of the molecular axes. To this end, it is useful to define an anisotropy ratio

$$R(t) = \frac{r_1(t)}{r_2(t)}. \quad (40)$$

If both  $r_1$  and  $r_2$  decays are single exponential with the same time constant, then the anisotropy ratio  $R(t)$  is a constant. On the other hand, if  $r_1$  or  $r_2$  exhibit multiexponential decay, then  $R(t)$  is not constant. Therefore, the anisotropy ratio

$R(t)$  can serve as an indicator to show whether the anisotropies are single exponential or multiexponential. From the anisotropies  $r_1$  and  $r_2$  in Eqs. (36) and (37), e.g., the anisotropy ratio is

$$R(t) = \frac{(1+3P^2)}{(2+P^2)} \frac{(1+\sqrt{3}C)[2+P^2+(9/\sqrt{3})PC] + (C-\sqrt{3})[(2+P^2)C - (9/\sqrt{3})P]e^{-\Delta Et}}{(1+\sqrt{3}C)[3P^2-1+(6/\sqrt{3})PC] + (C-\sqrt{3})[(3P^2-1)C - (6/\sqrt{3})P]e^{-\Delta Et}}, \quad (41)$$

where  $\Delta E$  is the difference between the rotational eigenvalues  $E_2^{(2)}$  and  $E_0^{(2)}$ . If  $R(t)$  is time dependent, then either the  $r_1$  or  $r_2$  decays (or both) must be double exponential. If  $R(t)$  is constant, both the  $r_1$  and  $r_2$  decays are single exponential (or double exponential in the special cases where  $P=0$ , or  $\pm 1$ ).

To determine whether  $R(t)$  is a constant, it suffices to know  $R(t)$  at two times— $R(0)$  and  $R(\infty)$  the initial value and the long-time value, respectively. The larger the difference between  $R(0)$  and  $R(\infty)$ , the more easily the two exponentials can be distinguished. The limiting values  $R(0)$  and  $R(\infty)$  are determined by the two molecular constants  $P$  and  $C$ . From Eq. (41), the limiting values are

$$R(0) = \frac{(1+3P^2)(2+P^2+9P)}{(2+P^2)(3P^2-1+6P)} \quad (42)$$

and

$$R(\infty) = \frac{(1+3P^2)[2+P^2+(9/\sqrt{3})PC]}{(2+P^2)[3P^2-1+(6/\sqrt{3})PC]}. \quad (43)$$

The value  $R(0)$  depends only on the constant  $P$ , reflecting the fact that the initial value does not depend on the rotational decay. Hence, the constant  $P$  can be determined from the value of  $R(0)$  in Eq. (42) and the constant  $C$  can be determined from the value of  $R(\infty)$  in Eq. (43).

If the difference between  $R(0)$  and  $R(\infty)$  is large, then  $R(t)$  will clearly show a time dependence. Figure 1 shows plots of  $R(0)$  and  $R(\infty)$  as a function of  $C$  with  $P=0.5$ . The values of  $R(0)$  and  $R(\infty)$  are distinct except at the point  $C=\sqrt{3}$ , which occurs for an oblate symmetric rotor (with

$D_z < D_x = D_y$ ). Figure 2 shows plots of the time dependence  $R(t)$  for (a)  $C=-1$  and  $P=0.5$ , and for (b)  $C=-1$  and  $P=-0.5$ . For both cases (a) and (b),  $R(t)$  is clearly time dependent and two exponential components can be determined. In contrast, if only  $r_1$  (say) were measured, the two exponential components would not be discerned, especially in case (a), since the relative contribution of the first component is quite small.

The separation of two exponential components in the anisotropy decays results in the measurement of two rotational eigenvalues  $E_0^{(2)}$  and  $E_2^{(2)}$ , and the constant  $C$ . In turn, three rotational diffusion constants  $D_x$ ,  $D_y$ , and  $D_z$  can be determined from  $E_0^{(2)}$ ,  $E_2^{(2)}$ , and  $C$ . This capability of two-photon anisotropy decay measurements could become useful in the study of rotational motion.

## V. DISCUSSION AND CONCLUSIONS

Time-resolved TPE fluorescence decays were introduced by Lakowicz and co-workers<sup>5-7</sup> as a potential method of generating initial anisotropies larger than 0.4, the maximum possible in conventional fluorescence anisotropy decay measurements. In limited circumstances, depending on the two-photon tensor, this is certainly possible. However, as we have shown in this paper, the value of anisotropic time-resolved TPE spectroscopy is not limited by any means to generation of large anisotropies. Rather, by measurement of two or more

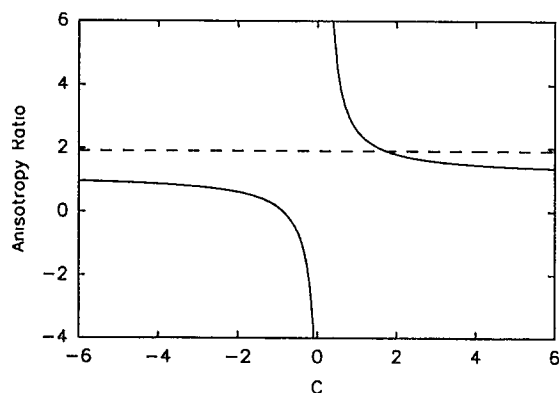


FIG. 1. The anisotropy ratio  $R(0)$  (dashed line) and  $R(\infty)$  (solid line) as a function of  $C$  with  $P=0.5$ . These parameters are defined in Eqs. (38)–(40).

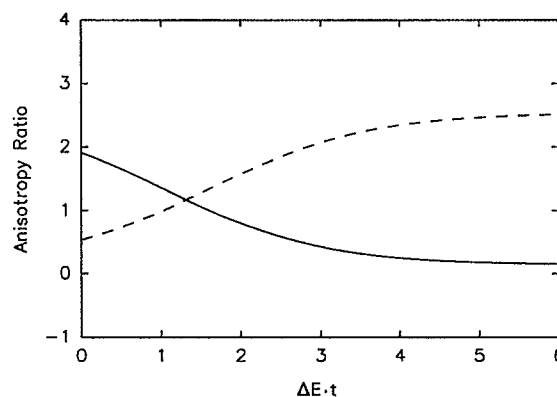


FIG. 2. The time dependence  $R(t)$  for (a)  $C=-1$  and  $P=0.5$  (solid line) and (b)  $C=-1$  and  $P=-0.5$  (dashed line). The time scale is normalized to the difference  $\Delta E$  between the rotational eigenvalues  $E_2^{(2)}$  and  $E_0^{(2)}$ .

anisotropy parameters, as well as the two-photon polarization parameter  $\Omega_p$ , details unavailable by conventional one-photon methods can be extracted.

The orientational average and polarization dependence of fluorescence induced by TPE were first treated over 20 years ago by McClain.<sup>2,10</sup> The orientational averages were carried out directly with Cartesian tensors. While this approach has appealing features, it also carries limitations due to the fact that the isotropic and anisotropic components of the signal are not separated. As a result, the effects of rotational motion are not readily incorporated into the theory, which therefore cannot be applied to rotating molecules (a fact that was recognized and pointed out by McClain<sup>10</sup>). In contrast, the spherical tensor formalism employed in Ref. 11 and in the present paper lends itself readily to the treatment of rotating molecules in liquid samples, since rotation affects the anisotropic, but not the isotropic, components, and these appear separately in the decomposition into spherical tensors.

When randomly oriented molecules are excited by two identical photons from a single laser beam, four independent parameters can be determined, as shown both in McClain's theory<sup>10</sup> and in Ref. 11. While McClain discussed only the case of a real two-photon tensor,<sup>10</sup> our treatment also shows that if two beams with different wavelengths are used, the number of independent parameters ( $Q$  space in Ref. 10) is increased from four to seven or nine for real or complex two-photon tensors, respectively. The complex two-photon tensor occurs when one of the two laser beams is absorbed by the sample due to a one-photon resonance.

The two-photon polarization ratio  $\Omega_p = \delta_{\text{cir}}/\delta_{\text{lin}}$  is a central quantity in determining the nature of the two-photon transitions.<sup>9,22,23</sup> This ratio is isotropic and depends only on the isotropic absorptivities  $\delta^{(L)}$ .<sup>11</sup> Anisotropic contributions must be excluded in order to measure  $\Omega_p$  correctly. Frequently, anisotropic contributions are assumed to be absent due to rapid rotational reorientation relative to the fluorescence lifetime. For samples where this assumption does not hold,  $\Omega_p$  can be determined from four measurements,  $\delta_{111,1}$ ,  $\delta_{222,1}$ ,  $\delta_{\text{cir},\parallel}$ , and  $\delta_{\text{cir},\perp}$ . Without an analyzing polarizer, such measurements are not possible. We have suggested an alternative method of determining  $\Omega_p$ , where only two measurements are required.<sup>11</sup> Briefly, this method involves the following "magic-angle" measurements: the linear two-photon absorptivity  $\delta_{\text{lin}}$  is determined by measuring the fluorescence with a polarization of 54.7° relative to the polarization of the pump beam. The circular two-photon absorptivity  $\delta_{\text{cir}}$  is determined by measuring fluorescence induced by a circularly polarized pump beam with a fluorescence polarization of 35.3° relative to the polarization plane of the excitation beam. The polarization ratio is then given by the ratio between these two magic-angle measurements.

In a recent paper,<sup>9</sup> Callis has applied McClain's formalism to derive relationships between the two-photon polarization ratio  $\Omega_p$  and the fluorescence anisotropy  $r_1$  induced by TPE for the case where two identical photons are absorbed. Using  $\Omega_p$  and  $r_1$ , it was possible to deduce the two-photon tensor shape and orientations for indole and 3-methyl indole. Semiempirical molecular orbital theory calculations of two-

photon anisotropy parameters also were presented which agree at least qualitatively with the experimental results. This approach permits an improved understanding of the two-photon polarization ratio  $\Omega_p$  and the two-photon anisotropy. However, it is of course subject to the same limitations that characterize McClain's formalism and therefore can be applied only to systems where rotation is restricted, or to the initial  $t=0$  signal.

To summarize, we have developed the theory of the time-resolved anisotropic fluorescence and transient absorption induced by two-photon excitation (TPE) for a randomly oriented molecular system. The time dependence due to rotational diffusion of the orientational distribution function induced by TPE has been obtained. Since the inversion symmetry of the system is conserved in the TPE process, the orientation distribution function contains only the terms of even rank  $f^{(0)}(\Omega, t)$ ,  $f^{(2)}(\Omega, t)$ , and  $f^{(4)}(\Omega, t)$ . However, only the  $L=0$  and 2 distributions are detected by TPE of fluorescence or transient absorption.

The time dependence of the fluorescence or transient absorption excited by TPE is then obtained from the time dependence of the orientational distribution function. The signal consists of both isotropic and anisotropic components. The isotropic component responds solely to the population decay, while the anisotropic component is sensitive to rotational decay as well. The anisotropic decay in general contains up to five exponentials, and is similar in that respect to anisotropic fluorescence induced by one-photon excitation. However, additional anisotropy parameters can be measured by the TPE technique. Two anisotropies—the linear anisotropy  $r_1$  and the circular anisotropy  $r_2$ —can be measured by TPE with a single laser beam. In general,  $r_1$  and  $r_2$  differ from one another. We have defined an anisotropy ratio  $R(t)$ , the ratio of  $r_1$  to  $r_2$ , as an indicator of multiple exponential decay of  $r_1$  and  $r_2$  since  $R(t)$  must be a constant if  $r_1$  and  $r_2$  are single exponential with the same rotational time constant.

An important potential application of time-resolved anisotropic two-photon spectroscopy involves determination of the rotational diffusion coefficients from measurements of  $r_1$  and  $r_2$  anisotropy decays. The additional information available from TPE can in some cases lead to resolution of rotational motion about the principal diffusion axes of the molecule. These components of rotational motion have not been resolved in the vast majority of rotational measurements following one-photon excitation.

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